



## Surveying Pathogenic Bacteria in Vegetables

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# Surveying Pathogenic Bacteria in Vegetables

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## INTRODUCTION

Outbreaks of food-borne disease have increasingly been linked to the consumption of vegetables. Consumers' demands for fresh and minimally processed fruits and vegetables are increasing, and at the same time potential sources of pathogen infestation are increasing due to the more frequent use of animal manures as fertilizers in organic versus conventional farming. On the other hand, a higher antagonistic potential against invading pathogens is implicated by the more diverse microbiota in organic soils.

Vegetable-associated outbreaks in Europe are not well documented, and guidelines are missing for reducing risks of pathogen infestation. Thus, a survey on (mainly) organically grown vegetables has been carried out by the **PathOrganic** consortium in five European countries as a basis for elaborating recommendations regarding food safety measures.

## METHODS

Manures and slurries of various animal origin and treatment used for fertilizing vegetable fields were analyzed for the presence of pathogenic *E. coli*, *Salmonella* sp., *Staphylococcus aureus*, *Listeria* sp. and *Campylobacter* spp. Fields where manure screening results suggested a potential risk of pathogen transfer to vegetables were selected for a broad-scale survey on vegetables mainly from organic production.

4620 plants of lettuce, 1900 plants of spinach and 500 carrots were collected from 14 fields in five countries; and were then processed in batches of 10 plants for pathogen specific enrichment cultures and microbial DNA isolation. PCR based methods were used to test the samples for the prevalence of the same pathogens as analyzed in manures. Common protocols were used regarding harvest and sample preparation in the various labs; and molecular analyses were shared according to expertise. At the same time, information on farm management practices was collected via questionnaires for the evaluation of risk factors.

## RESULTS

A higher incidence of pathogen infestation of manures and vegetables was seen in the screenings than expected. Relationships of vegetable infestation with manure contamination, however, are not straight-forward and need further exploration. The significance of specific farm management practices for risks of pathogen infestation is currently investigated in various experimental trials and through the evaluation of questionnaires filed out by farmers.

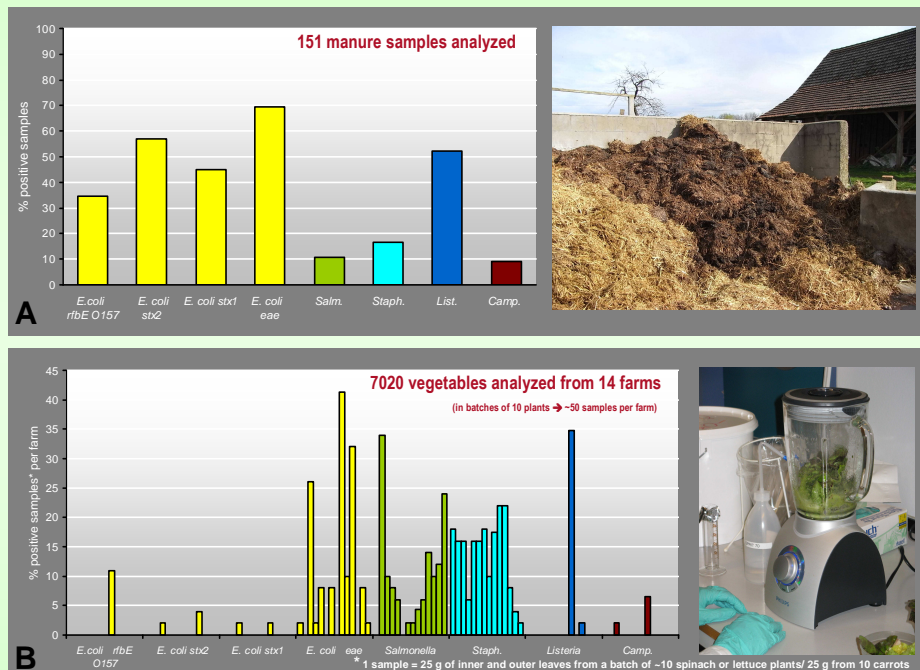


Figure 1. Results of the PCR-based screenings of (A) 151 animal manure samples (from vegetable farms) and (B) 696 vegetable samples (from 14 farms, given per individual farm) in % positive PCRs for the pathogen specific marker gene. Please note the different scalings of the x-axes. Marker genes analyzed: *rfbE* (*E. coli* O157), *stx1*, *stx2* and *eae* virulence genes (*E. coli* and others), *invA* (*Salmonella* sp.), *nuc* (*S. aureus*), *iap* (*Listeria monocytogenes*), 16S rRNA of *Campylobacter* spp. targeted by primers CampCJL1F and CaArHeREV.

## CONCLUSIONS AND OUTLOOK

- High baseline levels of bacterial pathogen infestation were found both in manures and in vegetables. The significance of the findings for potential food safety risks is analyzed in ongoing studies.
- Effects of environment and management related factors on the colonization behavior and persistence of pathogens in non animal/ human host environments are presently studied in greenhouse and field experiments.
- Bacterial pathogens isolated from vegetables will be analyzed and compared to animal/ human host isolates regarding pathogenicity traits.
- Survey results and information obtained via questionnaires together with data from experimental trials will feed into a microbiological risk assessment model.